Griffith :-

Studies in uric acid metabolism in man.

STUDIES IN URIC ACID METABOLISM IN MAN

Variations in the Uric Acid Content of Normal Human Saliva

BY

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THESIS

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Wendell Horace Griffith

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Table of Contents

I

General Introduction

Boucherson (1) first reported the presence of uric acid in the secretion of the salivary glands in 1885. Not having a quantitative micro method, he attempted to determine variations in the excretion of uric acid by means of the murexide test. He believed that the salivary glands excreted uric acid only when it was in excess in the blood and that this process was abnormal and did not take place when the glands were performing the normal function of ptyalin secretion. Stocker (2) reported an increase in uric acid in saliva in almost all cases in which there was an increased content of uric acid in the blood. v. Noorden and Fischer, (3) using the Folin-MaCallum reagent, found that the average excretion of uric acid in adults was 10 mg. per 100 cc. and that this was increased in gouty conditions. In young people the values for salivary uric acid were considerably lower, 1-2 mg. per 100 cc. Lowenstein and Gies (4) found an average of 2.1 mg. in men and 1.11 mg. per 100 cc. in women. They considered that for normal individuals the uric acid is independent of the diet but is influenced by the rate of secretion as well as by the nature of the stimulant employed to increase the flow of saliva. They found that variations in endogenous metabolism of uric acid were registered promptly by the salivary uric acid. They obtained an immediate rise after increased muscular exertion and after ingestion of purine-free food following a brief fast. They also observed a definite relationship between the quantities of uric acid in the saliva and in the urine. Unfortunately, only a brief report of the work of Lowenstein and Gies has been published up to this time so that the details and method of their work were not at hand.

II

Purpose of Experiments

The following experiments were carried out in order to obtain additional figures for the average content of uric acid in saliva and the variations in the amount excreted due to the various factors of diet and stimulus. According to the work of Lewis, Dunn, and Doisy, (5) the increased excretion of endogenous uric acid in the urine after the ingestion of purine-free protein is due to a general stimulation of all cellular metabolism by amino-acids, the products of the digestion of protein, and not due to the secretory activity of the digestive tract as suggested by Mares. If the salivary glands are organs of excretion and pick out uric acid from the blood, it would seem that the amount present in the saliva would be considerably more than 1-2 mg. per 100 cc. On the other hand, if the ingestion of protein increases the general cellular metabolism, then a slight rise in the salivary uric acid would be expected as a result of the increased metabolism of those glands following ingestion of protein food. If the source of the salivary uric acid is the metabolic activity of the glands then there should be a rise in the salivary uric acid whenever the glands are stimulated.

The salivary secretion, for the most part, comes from the three pairs of glands, the parotid, the submaxillary, and the sublingual. Of these, the parotid is a serous or albuminous gland and its secretion is a thin watery liquid containing little or no mucin, most of the ptyalin, and some protein. The cellular granules, which disappear on continued secretory activity, are protein granules. The sublingual gland is a mucous gland and secretes a thick viscid saliva. The submaxillary gland is both serous and mucous with a secretion similar to that of the sublingual. The granules in the mucous glands are mucin granules. The sublingual saliva (6) contains the highest percentage of solids, 2.75 per cent; the submaxillary saliva, 2.3 per cent; and the parotid saliva, only 0.3 to 0.5 per

cent. The mixed saliva averages about 0.5 per cent solids.

Saliva is formed from the protoplasm of the cells of the glands with the admixture of salts, water, and some other substances derived from the blood. It is really transformed protoplasm. During the time of active secretion, raw material is brought to the gland by the blood stream since vasodilation takes place at that time and the flow of blood to the glands is greatly increased. Out of this raw material protoplasm is formed which is transformed into granules of mucin and protein and the other salivary constituents. These accumulate in the glands and are discharged during secretion. There is no reason to believe that changes in the cell at the end of a day's active secretion differ from the changes in the first ten minutes, though the changes may be accelerated by certain stimuli. The glands are called upon to do an enormous amount of work daily in comparison to their size. The total weight is not more than 60-70 grams, yet they secrete about 1500 cc. per day. The oxygen consumption (7) per gram in the active state is about 0.028 cc. per hour. The muscles (7) use only 0.004 cc. per gram per hour under similar conditions. In view of this marked metabolic work, a rise in the salivary uric acid might be expected whenever the glands are stimulated to increased activity.

III

Methods

For the determination of the uric acid, the Benedict and Hitchcock modification of the Folin-MaCallum-Denis procedure was used with one or two slight variations. It was found impossible to obtain uniformly constant results using the technique as described by Benedict and Hitchcock, due to changes in the color of the solution. They prepared the standard as follows. 5 cc. of the standard uric acid solution, contained in a 50 cc. volumetric flask, are treated with 2 drops of 5 per cent KCN, 2 cc. of uric acid reagent, 10 cc. of 20 per cent Na_2CO_3 and

diluted to the mark at the end of one-half minute. This is made up at the same time as the sample and the comparison is made at once. Bogert (8) found that the standard color did not reach a maximum until 15 minutes after dilution, whether the solution stood one-half minute before dilution or not; and, she advised dilution of the sample with 20-30 cc. of water before the addition of the carbonate in order to prevent turbidity. Bogert also found that the sample faded faster than the standard and usually developed the maximum color in a shorter time. She recommended that the standard be allowed to stand 15 minutes after dilution before reading but that the sample be placed in the colorimeter cup at once after dilution and readings made every few minutes until it reached a maximum. In this case it would not be possible to develop the standard color and the unknown color at the same time.

Numerous determinations were made on the standard solutions for the purpose of testing the above results. The following experiment shows the time necessary for the development of the maximum color in the standard. Two standard solutions were treated according to the Benedict-Hitchcock procedure and compared at once. One was left in the cup and compared at intervals with similarly treated fresh standards.

Fresh Standards	Old Standard	After
mm.	mm.	
15	15	3 min.
15	13.2	8 "
15	13.2	13 "
15	13.2	19 "
15	13.7	39 "

In this instance, the standard reached a maximum in eight minutes and remained constant for at least twenty minutes.

The following experiment shows the variation in a fresh sample when compared with a standard which had stood 13 minutes before reading. This shows a

maximum color development in 6 1/2 minutes and a constant color for at least 30 minutes. Therefore, in the subsequent determinations, the standard was allowed to stand 8-10 minutes after dilution before reading.

Standard	Sample	After
mm.	mm.	
15	18.6	1 1/2 min.
	17.6	2 1/4 "
	17.1	2 3/4 "
	16.4	4 "
	16.2	4 1/2 "
	15.7	5 "
	15.4	6 "
	15.0	6 1/2 "
	15.1	7 1/2 "
	14.9	13 "
	15.1	19 "
	14.9	31 1/2 "

It was found impossible to recover the full amount of uric acid when a sample of the standard solution was treated as in the regular Benedict-Hitchcock procedure, if the sample was transferred to the flask from the centrifuge tube with 20-30 cc. of water before addition of the carbonate, as recommended by Bogert. The following experiments show this. In each, the standard was prepared according to the Benedict-Hitchcock procedure but allowed to stand about ten minutes before reading. Both Sample I and Sample II contained the same amount of uric acid as the standards. Sample I was diluted with 25 cc. of water before addition of the carbonate. The carbonate was added directly to Sample II in the centrifuge tube. Sample II developed as much color as the standard but Sample I did not. Dilution of the sample before addition of the carbonate was therefore abandoned.

Standard	Sample I	After
mm.	mm.	
15	19.1	1 1/4 min.
	18.2	2 "
	17.8	3 "
	17.2	4 1/4 "
	16.3	6 "
	15.9	9 "
	16	10 1/4 "

Standard	Sample II	After
mm.	mm.	
15	15.6	2 1/2 min.
	15.6	5 "
	14.9	7 1/2 "
	15.3	9 "
	15.0	9 1/2 "

It was necessary to find some means of removing the protein completely from the saliva as is necessary in the determination of uric acid in blood. Most of the protein can be removed by boiling the slightly acidified saliva and filtering; but, enough protein remains to interfere with the subsequent colorimetric reading. It has been shown by Bogert (8) that the use of colloidal iron results in a loss of some uric acid. In the present experiments, alumina cream was found to remove the uncoagulable protein satisfactorily and yet not interfere with the uric acid determination. The alumina cream was prepared by adding NH_4OH to a saturated solution of commercial alum until slightly alkaline to litmus and then adding dilute H_2SO_4 until the solution was faintly acid.

Considerable difficulty was encountered in finding a quick and suitable method of filtration. Even with the use of the alumina cream, direct filtration through an ordinary funnel was too slow to be satisfactory. The most satisfactory method involved the use of the Buchner funnel and suction. The complete method as finally determined upon is as follows. The saliva is made slightly acid to litmus with dilute acetic acid and heated to boiling to coagulate the proteins. It is then transferred to a 50 cc. volumetric flask and about 2 cc. of alumina cream are added. The solution is made up to volume with distilled water, thoroughly shaken, poured into a Buchner funnel, and filtered through two layers of filter paper, using moderate suction. The first few drops of filtrate are usually turbid, and, as soon as the filtrate becomes clear, it is necessary to transfer the funnel to a second flask, pouring the contents of the first flask back into the funnel.

The whole procedure takes only a few minutes and 45-48 cc. of the original 50 cc. can be recovered. This is evaporated down to 5-6 cc. in a small beaker and transferred quantitatively to a centrifuge tube, using as little water as possible. This solution is treated according to the regular Benedict-Hitchcock procedure with the exception noted above. In the later experiments, the standard was treated the same as the sample; i.e., precipitation with the ammoniacal silver lactate, etc. In this case the comparisons were made immediately after dilution. If the color of any sample was too weak to warrant dilution to 25 cc., the solution was left in the centrifuge tube and diluted up to the 15 cc. mark. The samples were very seldom turbid after addition of carbonate. If turbidity developed, the solutions were centrifuged before reading. It was noticed that turbidity was more frequent when the reagents were old. The following typical determinations, carried out as above described, show the recovery of added uric acid.

I

50 cc. of saliva plus 0.2 mg. uric acid----------	1.27	mg. per 100 cc.
50 cc. " " " " " " " ----------	1.27	" " " "
75 cc. " " ----------	0.87	" " " "
75 cc. " " ----------	0.91	" " " "
mg. uric acid added per 100 cc.----------	0.40	
mg. uric acid recovered per 100 cc.----------	0.38	

II

20 cc. of saliva plus 0.2 mg. uric acid----------	1.68	mg. per 100 cc.
47 cc. " " ----------	0.66	" " " "
mg. uric acid added per 100 cc.----------	1.00	
mg. uric acid recovered per 100 cc.----------	1.02	

III

20 cc. of saliva plus 0.2 mg. uric acid----------	1.50	mg. per 100 cc.
40 cc. " " ----------	0.54	" " " "
mg. uric acid added per 100 cc.----------	1.00	
mg. uric acid recovered per 100 cc.----------	0.96	

IV

Bacterial Decomposition of Uric Acid

At the beginning of these experiments it was not convenient to make the determinations in the forenoon, so it was customary to collect saliva at certain periods during the forenoon, and run the determinations in the afternoon. Thus, the saliva was allowed to stand without preservative at the room temperature for 3-4 hours. It was observed that the morning saliva sometimes contained no uric acid and always contained less than the saliva collected after dinner. It was assumed at first that this was normally the case. However, the frequent absence of uric acid in the morning saliva suggested that possibly the uric acid was destroyed on standing at room temperature for a few hours. Determinations were made on portions of samples which were allowed to stand without preservative and it was found that the amount of uric acid decreased rapidly. This explained the low morning figures and the low figures obtained when morning and afternoon salivas were mixed. The following experiments show this destruction of uric acid.

Saliva Without Preservative

	Uric acid in mg. per 100 cc.		
Time elapsing after collection of sample	0	3-4 hrs.	24 hrs.
Sample I	1.2	none	
Sample II	1.8	0.68	none
Sample III	1.1	none	

This destruction of uric acid might have been due to bacterial decomposition or to the presence of an enzyme, a uricase, in the saliva. If this were due to bacterial decomposition, preservatives added to the saliva should inhibit the bacterial action and sterilization by means of a Berkfeldt filter should completely prevent any destruction. If neither of these procedures prevented the

loss of uric acid, then it would follow that the uric acid was destroyed by an enzyme.

25 cc. of the uric acid standard, containing 5 mg. of uric acid, were added to 75 cc. of saliva which was then divided into two portions. One portion was allowed to stand exposed at room temperature, the other was shaken with toluene and chloroform. Uric acid determinations were made on the two samples at intervals as follows:

Comparison of the Uric Acid Content of Saliva with and without Preservatives

	Uric acid in mg. per 100 cc.			
After	0	24 hrs.	48 hrs.	72 hrs.
Saliva with Preservatives	4.90	4.90		4.88
Saliva without Preservatives	4.90	1.20	none	

This shows no destruction of uric acid after 72 hours in the saliva containing the preservatives, but a marked decomposition is indicated in the saliva without preservatives.

Another sample of saliva was filtered through a Berkfeldt filter in order to remove the bacteria. This saliva was first treated with alumina cream in the cold and filtered in order to facilitate the final filtration through the Berkfeldt. Some of the uric acid standard was added to the filtrate so as to increase the amount of uric acid present. Uric acid determinations were made at intervals on the filtrate. No growth was obtained on plates poured from agar inoculated with 1 cc. of the sterilized filtrate.

Sterilized Saliva

After	0	19 hrs.	46 hrs.
Uric acid in mg. per 100 cc.	4.57	4.65	4.70

These experiments showed clearly that uric acid was destroyed in saliva which was allowed to stand without preservative; and, that the destruction was due to bacterial decomposition and not to an exogenous uricase in the saliva. To check this bacterial action, all subsequent samples of saliva were acidified and boiled vigorously as soon as they were collected.

V

Normal Individual Variations in Salivary Uric Acid

In order to find the variations in the amount of uric acid normally present in the saliva, determinations were made on samples of saliva from ten individuals. A sample was collected from each subject on two different days but all the samples were collected at the same hour, about 11 a.m., thus making the results more nearly comparable.

In all cases, acceleration of the rate of secretion of saliva was obtained by chewing pure paraffin. The saliva collected in this manner was thin and watery and was very similar to parotid saliva in appearance. In order to control variations due to differences in dilution, total solids were determined on each sample by evaporating 5 cc. to constant weight in a crucible. 25 cc. of saliva were used in each uric acid determination. With paraffin as a stimulus, it usually required about 30 minutes to collect the necessary 30 cc. for the two determinations.

Variations in the Uric Acid of Normal Salivas

Subject	Uric Acid mg. per 100 cc.	Solids gm. per 100 cc.
1. G. J. C.	1.55	0.64
	1.69	0.62
2. G. D. G.	3.00	0.43
	2.54	0.34
3. W. H. G.	1.20	0.95
	1.10	0.92
4. D. V.	1.86	0.59
	1.87	0.42
5. H. W. H.	0.65	0.56
	0.70	0.82
[1]6. G. S.	0.88	0.71
	0.39	0.78
7. E. C. H.	2.67	0.67
	2.10	0.43
8. G. T. M.	2.20	0.52
	1.67	0.62
9. J. W. K.	2.10	0.57
	1.14	0.89
10. J. S.	1.42	0.48
Maximum amount	3.00	
Minimum amount	0.39	
Average amount	1.61	0.63

[1] Woman

Average solids for ten samples having over 1.61 mg. of uric acid per 100 cc. ---------- 0.52 gm. per 100 cc.

Average solids for nine samples having less than 1.61 mg. of uric acid per 100 cc. ---------- 0.75 gm. per 100 cc.

Average solids for six samples having highest amount of uric acid ---------- 0.49 gm. per 100 cc.

Average solids for six samples having lowest amount of uric acid ---------- 0.78 gm. per 100 cc.

The average amount of uric acid present, 1.61 mg. per 100 cc., was somewhat lower than the average found by Lowenstein and Gies and considerably lower than the figures given by v. Noorden and Fischer. The extremely low figures from subjects No. 5 and No. 6 decreased the average from 1.87 to 1.61 mg. per 100 cc. No. 6 was a woman on a purine-free diet and according to Lowenstein and Gies (4) the saliva of women normally contains less uric acid than that of men. There was no explanationfor the low content of uric acid in the saliva of No. 5. There was no wide variation between the two samples collected from each individual and it appeared that certain salivas were normally always higher or lower in their uric acid content than others -- at least for that particular time of the day.

VI

Normal Daily Variations in Salivary Uric Acid

In order to determine the normal excretion of uric acid throughout the day with one subject, samples were collected on several days at intervals of an hour or two. It was expected that an increase would be found immediately after each meal since the salivary glands are actively secreting then. Some of the results are shown in the following curves. (See Charts I and II). It was very evident that the excretion of uric acid was not uniform and it was equally clear that the rises were not due to the increased activity of the glands during meals since rises were just as common before as after a meal. This was especially true of the early morning saliva. Rises were almost invariably found immediately after breakfast. (See Tables I, II, and III). However, if two samples were collected before breakfast, the second showed a marked rise while the sample collected after breakfast on those days showed no such rise. (See Tables IV and V). No adequate explanation could be found for these variations. The rises before meals showed that the differences were certainly not due to the diet. It was hardly conceivable that the blood uric acid increased and decreased with such irregularity. These experi-

ments oppose the theory that the salivary uric acid is a measure of the blood uric acid. Evidently there are certain factors governing the metabolic activity of the salivary glands whose influence could not be controlled in these experiments.

Table I (See Chart I Curve I)

Saliva Collected	Uric Acid mg. per 100 cc.	Solids gms. per 100 cc.
6:00-7:00 a.m.	1.37	1.08
Breakfast 7:10-7:25		
7:30-8:00	1.75	0.93
11:00-11:30	0.935	0.77
Dinner 12:30 p.m.		
12:50-1:20	2.06	0.53
2:15-2:45	1.12	0.49
3:30-4:00	1.36	0.71
[1] 4:30-5:30	1.1	0.98

[1] Acid stimulus

Table II (See Chart I Curve II)

Time	Uric Acid mg. per 100 cc.	Solids gms.per 100 cc.
6:30-7:00 a.m.	1.18	0.99
Breakfast 7:20		
7:40-8:00	1.56	0.85
8:30-9:00	1.77	0.70
9:30-10:00	1.14	0.61
11:00-11:20	1.30	0.78
Dinner 11:45		
12:05-12:25 p.m.	1.67	0.69
1:15-1:45	1.10	0.60
2:30-3:00	1.26	0.60
3:00-3:30	1.16	0.62

Table III (See Chart I Curve III)

Saliva Collected	Uric Acid mg. per 100 cc.	Solids gms.per 100 cc.
5:50-6:20 a.m.		0.94
7:00-7:30	1.27	0.56
Breakfast 7:40		
7:50-8:10	1.28	0.63
11:00-11:30	1.35	0.75
Dinner 12:20 p.m.		
12:45-1:15	1.70	0.59
1:50-2:20	1.90	0.60
3:05-3:45	1.79	0.70
4:30-5:00	0.89	0.43

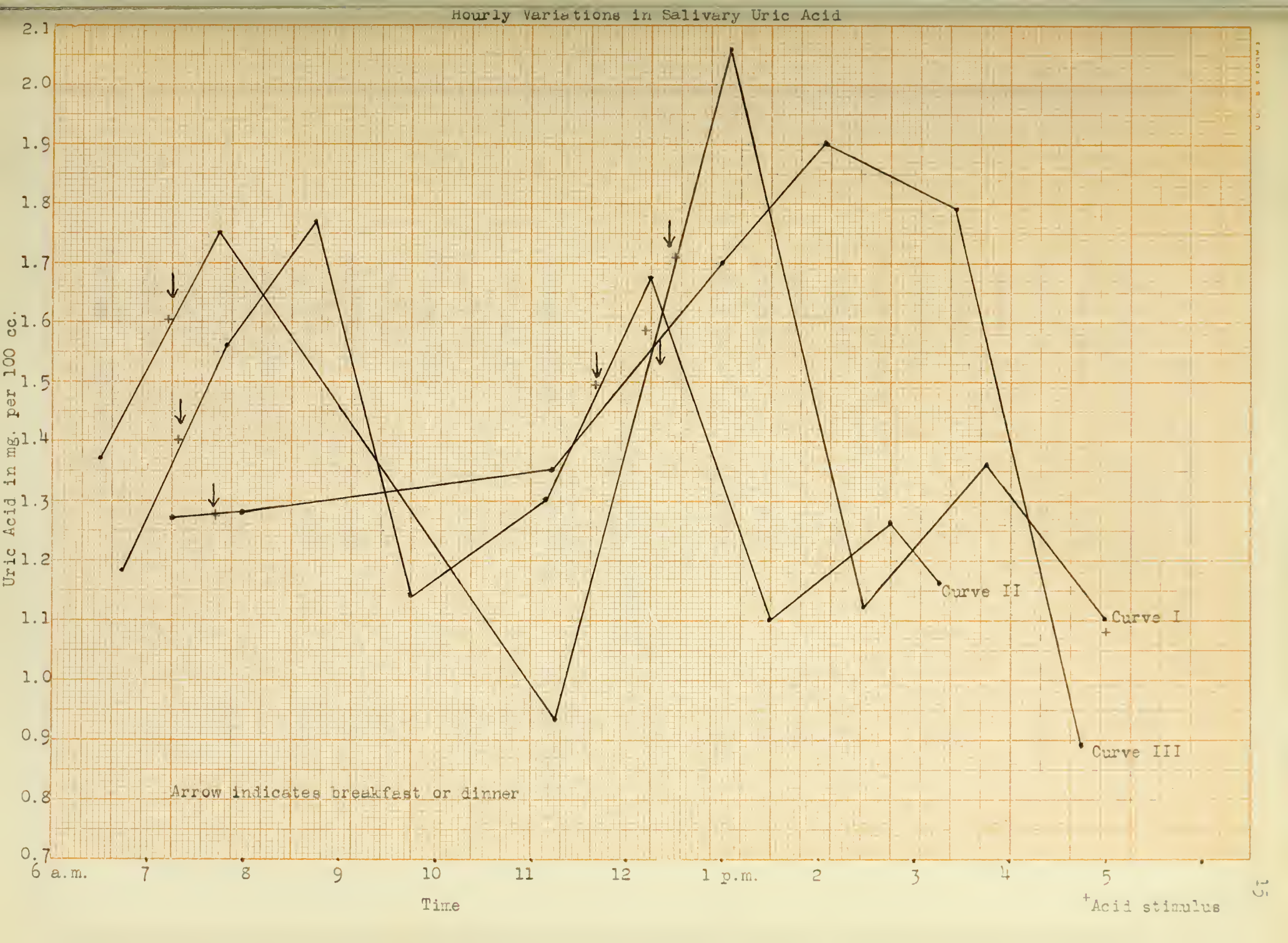
Hourly Variations in Salivary Uric Acid
Uric Acid in mg. per 100 cc.
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
6 a.m.
7
8
9
10
11
12
1 p.m.
2
3
4
5
Time
+Acid stimulus
Curve II
Curve I
Curve III
Arrow indicates breakfast or dinner

Table IV (See Chart II Curve IV)

Saliva Collected	Uric Acid mg. per 100 cc.	Solids gm. per 100 cc.
5:45-6:30 a.m.	0.78	0.72
6:35-7:05	1.24	0.496
Breakfast 7:20		
7:30-8:00	1.05	0.60
11:00-11:30	1.00	0.77
Dinner 12:15-12:30		
1:15-1:45	1.02	0.632
[1]2:20-2:50	1.45	0.69
3:00-3:30	0.70	0.53
[1]3:45-4:10	0.70	0.636

[1]Acid stimulus

Table V (See Chart II Curve V) (Also Chart III Curve V)

Saliva Collected	Uric Acid mg. per 100 cc.	Solids gm. per 100 cc.
7:10-7:30 a.m.	1.14	0.67
7:30-8:15	1.82	0.52
[1]Glycocoll 8:30		
9:00-9:30	1.30	0.71
10:00-10:30	1.30	0.55
11:00-11:30	1.58	0.65
Dinner 12:05 p.m.		
12:45-1:15	1.60	0.65
2:00-2:30	1.00	0.69
3:00-3:40	1.08	0.67

[1]10 gms. in 400 cc. water


Hourly Variations in Salivary Uric Acid
Uric Acid in mg. per 100 cc.
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
Arrow indicates breakfast or dinner
Curve V
Curve IV
+ Acid stimulus
6 a.m.
7
8
9
10
11
12
1 p.m.
2
3
4
5
Time

VII

Effect of Ingestion of Glycocoll on the Salivary Uric Acid

Ingested protein causes an increase in the endogenous uric acid excretion. Lewis, Dunn, and Doisy (5) have shown that the ingestion of 10 gms. of glycocoll caused a marked rise in the urinary uric acid within two hours. If the salivary uric acid registers promptly the rise in endogenous uric acid as suggested by Lowenstein and Gies, then a rise was to be expected in the salivary uric acid within two hours after the ingestion of glycocoll. On two days 10 gms. of glycocoll were ingested in place of the usual breakfast. In neither case was there a rise in the salivary uric acid within two hours and a half. (See Tables V, VI, and VII). The curves correspond to the curve[1] showing the variations during the morning and forenoon when no food had been ingested since the previous evening.

Table VI (See Chart III Curve VI)

Saliva Collected	Uric Acid mg. per 100 cc.	Solids gm. per 100 cc.
6:30-7:00 a.m.	1.24	0.65
7:45-8:10	1.61	0.51
[1]Glycocoll 8:40		
9:30-9:50	1.29	1.23
10:15-10:45	1.36	0.52
11:15-11:45	1.31	0.56
Dinner 12:05 p.m.		
12:45-1:15	1.15	0.56

[1] 10 gms. in 400 cc. water

[1] See Curve VII.

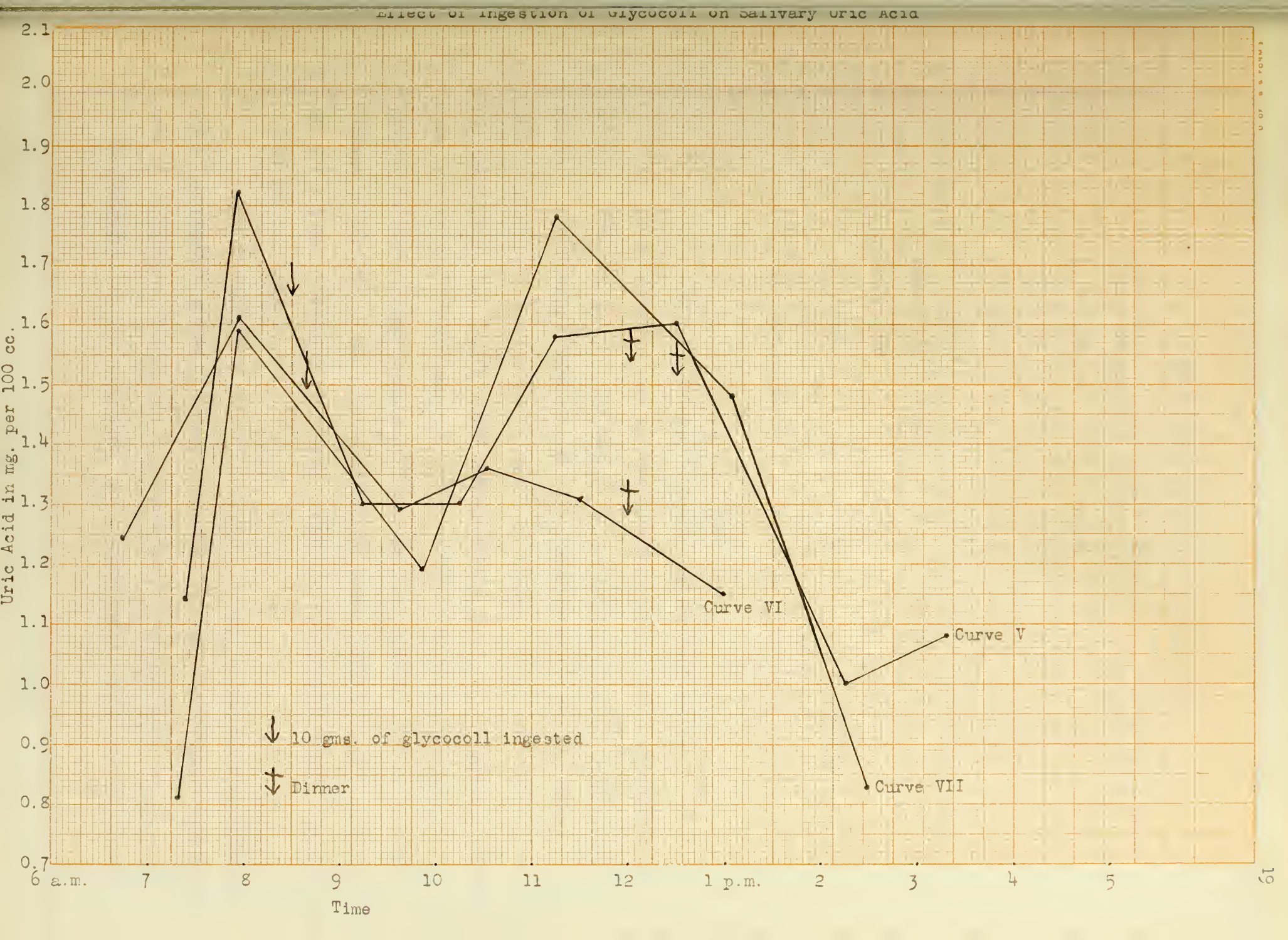
Effect of Ingestion of Glycocoll on Salivary Uric Acid
Uric Acid in mg. per 100 cc.
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
6 a.m.
7
8
9
10
11
12
1 p.m.
2
3
4
5
Time
Curve VI
Curve V
Curve VII
10 gms. of glycocoll ingested
Dinner

Table VII (See Chart III Curve VII)

Saliva Collected	Uric Acid mg. per 100 cc.	Solids gm. per 100 cc.
7:10-7:30 p.m.	0.81	0.65
7:30-8:15	1.59	0.60
9:30-10:15	1.19	0.74
11:00-11:30	1.78	0.67
[1]Dinner 12:30-12:40 p.m.		
12:50-1:20	1.47	0.76
2:15-2:45	0.83	0.79

[1] One quart of milk

VIII

Relation between Salivary Uric Acid and Solids

It was observed that in general high uric acid was accompanied by low solids. This was shown by the figures on the normal variation of uric acid in saliva. The average amount of uric acid present was 1.61 mg. per 100 cc. The average solids for the ten samples having more than 1.61 mg. of uric acid per 100 cc. was 0.52 gm. per 100 cc. The average solids for the nine samples having less than 1.61 mg. per 100 cc. was 0.75 gm. per 100 cc. The average solids for the six samples having the largest amounts of uric acid was 0.49 gm. per 100 cc. The average solids for the six samples having the lowest amounts of uric acid was 0.78 gm. per 100 cc. These figures showed a probable relationship between the salivary solids and uric acid.

This relationship was not so evident in some of the daily experiments. Table II certainly showed no connection between uric acid and solids. The solids were at a maximum in the morning and decreased to a minimum level by the middle of the forenoon. On the other hand, the relationship of high uric acid and low

solids was indicated in Table IV in the second morning sample collected. The second sample after dinner was also high in uric acid but it did not show a decrease in the solids. This was probably due to the fact that acid was used as the stimulant and this always produced a saliva high in solids. In view of the fact that the mucous glands govern the amount of solids present in the saliva, it was thought that possibly the uric acid, since it appeared to be increased when the solids were low, might originate mainly in the serous parotid gland. Experiments undertaken to determine this were inconclusive.

IX

Relation between Salivary Uric Acid and Fatigue of the Glands

It was found impossible to decrease the amount of salivary uric acid below 0.8-1.0 mg. per 100 cc. by collecting samples throughout the day at half hour intervals. (See Tables I and II). Continuous chewing of paraffin for 2-3 hours did not reduce the uric acid content below this minimum. This seemed to show that there was a minimum excretion of uric acid which probably represented the normal metabolic activity of the working glands. There was also a minimum output, about 0.5-0.6 gm. per 100 cc., which could not be decreased by continuous secretion of saliva for 2-3 hours.

Effect of Continuous Stimulation on Salivary Uric Acid and Solids

	Time p.m.	Quantity Secreted	Uric Acid	Solids
Experiment I	2:35-3:40	100 cc.		
	3:40-4:00	30 cc.	1.0	0.79
Experiment II	1:30-3:30	100 cc.		
	3:30-4:00	30 cc.	1.17	0.55
Experiment III	12:30-3:45	120 cc.		
	3:45-4:15	30 cc.	0.84	0.66
	4:15-4:45	30 cc.	1.30	0.63

X

Relation between the Salivary Uric Acid and the Rate of Secretion

The rate of secretion of saliva could be greatly accelerated by placing a drop of acetic acid on the tongue. The saliva obtained in this way was very thick and viscid and contained considerable mucin. It was typical mucous saliva and in appearance did not at all resemble the thin watery saliva obtained by chewing paraffin. It was possible to collect a 30 cc. sample in this manner in 15 minutes, which was half the time required when paraffin was used as the stimulant. The uric acid determinations on this saliva were too variable to be conclusive or satisfactory. Many of the samples showed no uric acid at all and it was not known whether or not this was due to its absence in the mucous saliva. In all samples collected with acetic acid as the stimulant, the solids were from 0.2-0.3 gm. per 100 cc. higher than in saliva collected with paraffin.

Relation between Uric Acid and the Secretory Stimulant

Experiment I

Time	Stimulant	Uric Acid mg. per 100 cc.	Solids gm. per 100 cc.
2:35-2:55 p.m.	Acetic Acid	0.00	0.98
2:55-3:13	" "	0.00	0.87
3:14-3:29	Acid and Paraffin	0.00	0.96
3:38-4:05	Paraffin	1.00	0.79
4:09-4:23	Acid and Paraffin	0.00	1.04

Experiment II

12:30-1:00 p.m.	Acetic Acid	0.00	0.65
1:15-1:45	" "	1.06	0.66
2:00-2:35	" "	0.00	0.56
2:50-3:35	" "	0.00	0.58
3:45-4:15	Paraffin	0.84	0.66
4:15-4:45	"	1.30	0.63

Experiment III

The following determinations were made in order to ascertain whether the uric acid had been precipitated by the acetic acid used as the stimulant, since the saliva obtained in this manner was acid. The experiment was inconclusive. 50 cc. of saliva were collected with the acid stimulant and divided into two portions. The saliva was distinctly acid to litmus. One portion was made alkaline to litmus by the addition of Na_2CO_3 in order to dissolve any precipitated uric acid and then made slightly acid with acetic acid.

	Uric Acid mg. per 100 cc.
Portion unchanged----------	1.30
Portion made alkaline and reacidified----------	0.60

Pilocarpine ordinarily stimulates the salivary glands to a marked secretory activity. In order to ascertain the effect of this increased secretion, on the amount of uric acid present, pilocarpine was taken by mouth. In the first experiment, 3.0 mg. of pilocarpine sulphate in solution were taken and an hour later the same dose was repeated. No effect at all was produced and it was concluded that the dose was too small. The experiment was repeated the following day with a dose of 8.0 mg. The effect was hardly noticed. The rate of secretion was increased slightly but not enough to influence the salivary uric acid or solids.

Effect of Pilocarpine on the Salivary Uric Acid

Experiment I

Time	Uric Acid mg. per 100 cc.	Solids gm. per 100 cc.
Breakfast 7:45 a.m.		
8:05-8:30	1.21	0.69
8:40-9:00	0.82	0.61
[1]Pilocarpine 9:27		
10:10-10:27	1.07	0.64
[1]Pilocarpine 10:33		
11:14-11:30	0.74	0.63
12:15-12:36 p.m.	0.88	0.60

[1]3 mg. in 100 cc. water

Experiment II

Breakfast 7:45 a.m.		
[1]Pilocarpine 9:00		
10:03-10:18	0.00	0.65
10:45-10:57	0.00	0.61
11:40-11:55	0.89	0.58

[1]8 mg. in 100 cc. water

XI

Summary

1. Salivary uric acid may be accurately determined by a modification of the Benedict-Hitchcock colorimetric method.

2. Uric acid was destroyed by the bacteria of the saliva in from 3-4 hours if the saliva was allowed to stand at room temperature without preservative.

3. The amount of uric acid present in the salivas of ten healthy individuals varied from 0.4-3.0 mg. per 100 cc. The average value was 1.61 mg. per 100 cc.

4. The excretion of uric acid in the saliva varied from hour to hour and was not dependent upon the stimulation of the glands by ingestion of food.

5. The ingestion of glycocoll did not increase the salivary uric acid.

6. A high content of uric acid in the saliva was usually accompanied by a low content of solids.

7. Salivary uric acid was not decreased below a minimum of 0.8-1.0 mg. per 100 cc. by continuous stimulation of the glands for two to three hours.

XII

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